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DESIGN AND CHARACTERIZATION OF POLYHERBAL FORMULATION FOR HAIR GROWTH CONTAINING *ECLIPTA ALBA*, *HIBISCUS ROSA-SIENSIS*, *TRIGONELLA FOENUM-GRAECUM* LINN.

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Keywords:

Eclipta alba, *Hibiscus rosa-siensis*, *Trigonella foenum-graecum*, Minoxidil, Hair growth

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ABSTRACT: Gelling agents such as Carbopol 934 and HPMC K 100 were used in different concentrations for the preparation of Topical polyherbal extract gel formulations containing (%w/w) *Eclipta alba*, *Hibiscus rosa-siensis*, *Trigonella foenum-graecum* Linn. Prepared gel extract of various herbal ingredients were characterized by different parameters like gelling capacity, pH, Homogeneity, Grittiness, Extrudability, Spreadability, and Viscosity. Satisfactory results were obtained after determining the above physicochemical parameters. The release data of all designed polyherbal extracts were compared with the marketed product (2% Minoxidil) to examine marketing feasibility and formulation PG1 was shown similarity with Standard (2% Minoxidil). Hence, the designed formulation will be a good alternative for available marketed products with low cost.

INTRODUCTION: Alopecia is a widespread issue, having influenced both genders of all races to various degrees for whatever length of time that humankind has existed. It has been recommended that alopecia could adversely affect physiological life and confidence between both sexual orientations. Despite the fact that the symptom related to this medication has constrained its pharmacological advantages subsequently the medication of plant origin is the best alternative to the drug of synthetic one India is a storehouse of therapeutic plants. Herbs are additionally utilized for the beautification of the body and the development of different cosmetics.

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In the traditional system of medicine, a variety of natural origin medicaments shown activity for hair growth, but less research over it makes low scientific backing and hence little or no information regarding its use [1].

Minoxidil is basically synthetic in nature; a potent vasodilator and consider as a safe medication for long-term use. The hair growth cycle is basically divided into three major stages *viz*., Anagen, Catagen, and Telogen. Out of which anagen is an important stage and nearly 85% of hairs lying in this phase at any one time. The length of the anagen phase or growth phase lies between two to six years. Anagen phase extends to Catagen which may last for one or two weeks, in this phase, the hair follicle shrinks to about 1/6 of the normal length. Hair does not grow but stays attached to the follicle while the dermal papilla stays in a resting phase below, is nothing but the resting phase (Telogen), which follows the Catagen phase and normally lasts about 5-6 weeks.

After a detailed literature survey carried out on crude drugs reveals essential information regarding the selection of drugs for the formulation of a cosmetic formulation for the promotion of hair growth activity. Hence, the present study was aimed to evaluate the hair growth activity of polyherbal extract gel, which includes *Eclipta alba*, *Hibiscus rosa-siensis*, *Trigonella foenum-graecum* Linn. in various concentrations [2].

MATERIALS AND METHODS:

Materials: Sanjivani Aushadhalay, Ghatkopar, Mumbai supplied seeds of *Trigonella foenum-graecum* while leaves of *Eclipta alba* and Flowers of *Hibiscus rosa-siensis* were obtained from local, market.

Preparation of Extracts: Obtained materials from suppliers were dried by air drying method. Then the dried material has been powdered by using mixer grinder and sieved to obtain a coarse form of it.

Extraction was carried out by using Soxhlet Apparatus for derived coarse powder of *Eclipta alba,* Flowers *of Hibiscus rosa siensis* and Seeds of *Trigonella foenum-graecum* continuously with methanol as a solvent for 48 h. Produced methanolic extracts then transferred to reduced pressure at 40 ºC in a vacuum dryer where this concentrated extract is converted to dried form and stored in desiccators.

Preparations of Polyherbal Extract Gel: Above prepared dried extract (2 gm) was then transfer into a solvent mixture containing Propylene glycol, methanol, and distilled water, in the ratio 1:1:1.

The required amount of gelling agents such as Carbopol 934 / HPMC K 100 was weighed. Then, Gelling agents were transferred into the prepared solvent mixture (Propylene glycol, methanol, distilled water). Allow soaking overnight in the solvent mixture so that the polymer will swell completely. Preservatives, propylparaben was added in the above mixture. Prepared formulation was stir continuously for two hrs at 500 rpm. After 2 hrs speed was reduced so that no air entrapment should be there. Finally, adjust the pH was adjusted to neutral by using triethanolamine, which resulted in a consistent gel [3].

TABLE 1: FORMULATION COMPOSITION OF POLYHERBAL EXTRACTS GEL

S. no.	Ingredient	PG1	PG2
1	Polyherbal extract (% w/w) (*E.a.: H.r.s.: T. f.g.* 3:2:1)	2	2
2	Carbopol 934 (%w/w)	1	-
3	HPMC K 100	-	2
4	Propylene glycol	15	15
5	Methanol	30	30
6	Propyl paraben	0.5	0.5
7	Triethanolamine	0.4	0.4
8	Distilled Water	Upto100	Upto100

PG1: Polyherbal extract gel with Carbopol 934 PG2: Polyherbal extract gel with HPMC K 100

FIG. 1: PG1: POLYHERBAL EXTRACT GEL WITH CARBOPOL

FIG. 2: PG2: POLYHERBAL EXTRACT GEL WITH HPMC 934 K100

Evaluation of Polyherbal Extract Gel:

Preliminary Evaluation: Preliminary evaluation such as color, odor has been carried out by visual inspection method and by smelling the product respectively. Results were reported elsewhere.

Physico-chemical Evaluations:

Measurement of pH: Digital pH meter was used to determine the pH of the polyherbal extract gel. One gram of sample was dissolved in 100 ml of distilled water and stored for two hours then the

measurement of pH of each formulation was done and recorded [4].

Homogeneity: Homogeneity of created polyherbal extract gel has been completed by visual assessment after the gels have been set in the container. Appearance and presence of any aggregates was controlled by visual investigation [4].

Grittiness: A pinch of product is rubbed on to the skin and observe under magnifying glasses [5].

Extrudability Study: Decent gel expel ideally from the gel with slight pressure applied. The extrudability of polyherbal extract from aluminum collapsible cylinders was calculated utilizing commonly used cylinder filling machine. Aluminum collapsible cylinders loaded up with 10gm. gels were held between two clasps. A cylinder was compacted and the extrudability of the definition was determined as far as weight in grams required expelling a 0.5 cm. ribbon of gel in 10 sec [5].

Extrudibility = Applied weight to extrude gel from tube (in gm) / area (in cm^2)

Spreadability: It was determined by a wooden block and glass slide apparatus. An excess of gel sample 4 gm was placed between two slides and a 1000 gm weight was placed on an upper slide for few minutes to compress and uniformly spread the gel between the slides. A weight of 50 gm was placed on the pan. The time required to separate the two slides was taken as a measure of spreadability. It was calculated using the formula.

$$S = M.L / T$$

Where, S = Spreadability, M = Weight tied to the upper slide, L= Length of the glass slide, T = Time in seconds

The length of the glass slide was taken in cm and weight tied to the upper slide was taken as 50 gm throughout the experiment [5].

Viscosity: The estimation of the viscosity of the formulated gel was done with a Brookfield Viscometer MLVT115 using spindle no. 64 at spindle speed 30 rpm. The corresponding dial reading was noted t every speed [6-7].

Primary Skin Irritation Test by using Wistar Strain Male Albino Rat: All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethical Committee. Two Wistar strain male albino rats weighed 120-150 gm were selected for the study. Each rat was caged individually. Food and water were given during the test period 24 h. prior to the test. The hair from the back of each rat of 1 cm^2 was shaved on the side of the spine to expose sufficiently large test areas which could accommodate two test sites were cleaned with surgical spirit. 0.5 mg quantity of formulations PG1, PG2 was applied over the respective test sites of one side of the spine. The test sites were observed for erythema and edema for 48 h. after application[7].

Animals: Wistar strain male albino rats, weighing between 120-150 gm, were fed on a standard diet and water ad libitum. The animals were housed at room temperature (24°C ± 2°C) on a normal day-night cycle (0 6:00 – 18:00 h).

Treatment: Animals were divided into 4 groups of 6 rats each. Group I was served as control. Group II was treated as standard (2% Minoxidil solution). Group III was a topically applied PG1 formulation. Group IV was a topically applied PG2 formulation.

A skin irritation test was carried out on rats. When prepared Polyherbal extract Gel applied, did not show any erythema on the skin surface. Thus, the prepared formulations were considered safe for topical administration.

Application of Test Samples: Hairs on the dorsal side of the animals were removed using shavers to assure complete denual of 6 sq cm area. Finally, denuded skin was wiped off with surgical spirit. Equal quantity (0.5mg) of prepared Polyherbal extract Gels and 0.5 ml standard 2% minoxidil solution were applied to the denuded area of albino rats once a day for 30 days.

Statistical Treatment: Data are reported as mean ± SEM Statistical analysis of data was carried out by one way ANOVA comparing all test groups versus control followed by Dunnet's test using Graph pad Instat v 3.1 software.

***In-vivo* Hair Growth Analysis:** On completion of 30^{th} days treatment, the hair growth status of all the groups was observed visually and data were recorded.

Qualitative Hair Growth Study: Qualitative hair growth was evaluated by visual observation of two parameters:

1. Hair growth initiation time *i.e.* minimum time taken to initiate hair growth on denuded skin region.
2. Hair growth completion time *i.e.* minimum time taken to completely cover the denuded skin region with new hair completely.

Hair growth initiation time and Hair growth completion time was recorded for each group of animals and compared with positive control Mintop® (Standard 2% ethanolic solution of minoxidil) and control.

Quantitative Hair Growth Study: The method described by uno [11] was followed for the quantitative evaluation of formulation. Two rats from each group was authenticated after the 10 days, 20 days and 30 days of treatment, skin biopsies were taken from the shaved area and a specimen was preserved in 10% formalin. The specimen was fixed on paraffin wax and blocks prepared for microtomy. After fixation, vertical sections of the skin were cut with the help of a semiautomatic rotary microtome. The sections were stained with hematoxylin and eosin.

The number of hair follicles per millimeter area of skin and ratio of hair follicles in different cyclic phases, like anagen (growth phase) and telogen (resting phase) was determined microscopically [8].

Hair Length Analysis: The hair was plucked randomly from the shaved area of rats from each group. After the 30th day of the treatment, the length of ten hairs was measured and the average length was determined and compared with animals from the control group.

Hair Weight Analysis: After 30 days, the rats of all the groups were sacrificed by cervical dislocation. Dorsal skin area (1 sq. cm) with hairs and without hairs was cut and weighed with the high precision analytical balance. After measuring, hair weight was calculated by subtracting the weight of skin from the weight of skin with hair [9].

***In-vitro* Hair Growth Analysis Hair Follicular Analysis:** Wistar strain male albino rats hair follicles, isolated from the neonates, were used for the present study.

The neonates were sacrificed by cervical dislocation and the dorsal portion of the skin was dissected out and washed thoroughly in phosphate-buffered saline. The skin was cut into small segments and individually placed in a Petri dish containing phosphate-buffered saline. The skin pieces were chopped thoroughly until the intact follicles came out from the skin. The separated intact hair follicles were isolated using a fine Pasteur pipette in binocular microscope. Individual, freshly isolated hair follicles were placed in separate wells of 96-well plates containing 150µl of Dulbecco's Modified Eagles Medium. Finally, 1.5 µl of (0.01mg/ml) Polyherbal extract Gel in dimethyl sulfoxide (DMSO) was added to the corresponding wells and the plates were maintained at 37 ºC. After 24 h. and 72 h. of treatment the status of hair follicle length was measured using a binocular microscope equipped with an eyepiece measuring graticule [9].

RESULTS AND DISCUSSION:

Preliminary Evaluation: The Organoleptic Characteristics of polyherbal extract gels were evaluated. The results are summarized in **Table 2**.

Physicochemical Evaluation: The physico-chemical parameters of polyherbal extract gels were determined. The results are summarized in **Table 3**.

TABLE 2: EVALUATION OF ORGANOLEPTIC CHARACTERISTICS

S. no.	Parameters	PG1	PG2
1	Colour	Reddish yellow	Reddish-brown
2	Odour	Characteristic	Characteristic

TABLE 3: DETERMINATION OF GELLING CAPACITY, pH, HOMOGENEITY, GRITTINESS, EXTRUDIABILITY, SPREADABILITY, VISCOSITY

S. no.	Formulation Code	Gelling Capacity	pH	Homogeneity	Grittiness	Extrudiability	Spreadability gm, cm/sec	Viscosity (centipoises)
1	PG1	Soft Gel	7.29	+++	-	+++	23.74	16799
2	PG1	Soft Gel	6.82	+++	-	+++	23.55	13749

Indication: + Poor, ++ Good, +++ Excellent, -Absent

The pH of the formulations was in the range of 6.5 to 7.5 which lies in the normal pH range of the skin and would not produce any skin irritation. This may be due to the addition of base triethanolamine to the resultant gel. All formulations were found to be homogeneous and free of grittiness. Spreadability denotes the extent of the area to which the gel readily spreads on application to skin or affected parts. The spreadability value was found to be in range of 23. 55 to 23.74 gm.cm/sec. The viscosity of various formulated gels was measured using Brookfield viscometer (MLVT 115 Model) at room temperature. In the gel formulation, consistency depends on the ratio of solid fraction, which produces the structure to the liquid fraction. The viscosity of various formulated gels was found to be in increasing order as the concentration of Carbopol 934 and HPMC K 100 increases ranging from 13749 and 16799 centipoises.

Hair Growth Activity:

Primary Skin Irritation Test: Primary skin irritation test was conducted to evaluate the irritation by the prepared Polyherbal extract Gels on intact skin of rats. All of the prepared Polyherbal extract Gels did not show any sign of irritation *i.e.* no erythema / no edema was observed at the site of application till 48 h. This indicates that the prepared Polyherbal extract Gels were non-irritant on skin of rats. Thus, the prepared Polyherbal extract Gels were considered safe for topical administration.

Qualitative Hair Growth Study: The times taken for hair growth initiation and completion were observed on the shaved area at the end of the course in all the groups. As shown in Table 4, the initiation of hair growth was observed on 15th day in control, 6th day in Standard (2% Minoxidil), 7th day in a group- PG1, 10th day in a group- PG2. Similarly, the completion of hair growth was found on 27th day in control, 18th day in Standard (2% Minoxidil), 19th day in a group- PG1, 22nd day in a group- PG2 respectively. The prepared Polyherbal extract Gels (PG1, PG2) showed significant hair growth activity as compared to the control group

TABLE 4: EFFECT OF POLYHERBAL EXTRACT GELS ON QUALITATIVE ANALYSIS OF HAIR GROWTH

S. no.	Group	No. of Rats	Time taken to initiate growth (in days)	Time taken for complete growth (in days)
1	Control	6	15 ± 0.82	27 ± 0.97
2	Standard (2% Minoxidil)	6	06 ± 0.41**	18 ± 0.25**
3	PG1	6	07 ± 0.25**	19 ± 1.14**
4	PG2	6	10 ± 0.68*	22 ± 1.06*

All Values were expressed as mean ± SEM, n = 6 *P < 0.05 and **P < 0.01: Significance versus control.

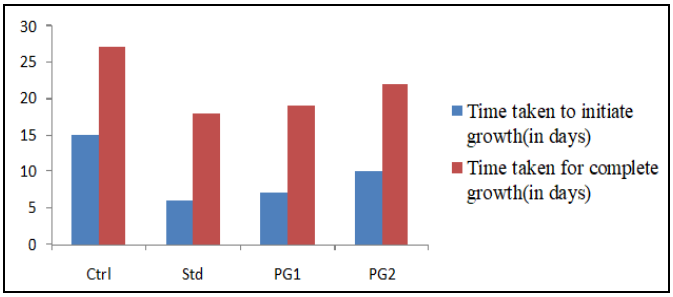

FIG. 3: EFFECT OF POLYHERBAL EXTRACT GELS ON QUALITATIVE ANALYSIS OF HAIR GROWTH

Quantitative Hair Growth Study: A considerable difference in cyclic phases of hair growth was observed in groups treated with Standard (2% Minoxidil) and Polyherbal extract Gels (PG1, PG2). Cyclic phases of hair growth were markedly affected by the Standard (2% Minoxidil) and Polyherbal extract Gels (PG1, PG2). After 30 days of treatment, the anagenic population of hair follicles in group control, Standard (2% Minoxidil), PG1, PG2 was found to be 51 ± 0.49, 65 ± 0.43, 63 ± 0.43 and 54 ± 0.52 respectively. Similarly, the telogenic population of hair follicles in group control, Standard (2% Minoxidil), PG1, PG2 was found to be 60 ± 1.20, 43 ± 0.50, 45 ± 0.35 and 56 ± 0.40 respectively. Thus, both polyherbal extract gel formulations in treated groups showed significant growth as compared to control.

The results are summarized in **Table 5**.

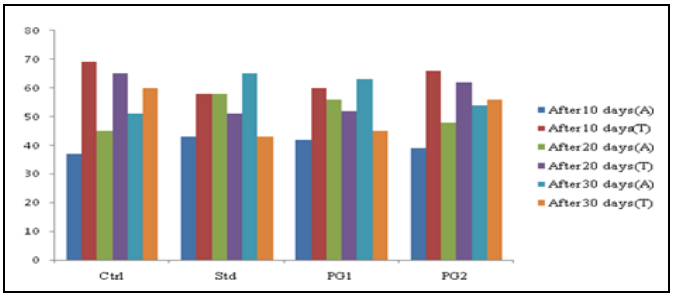

FIG. 4: EFFECT OF POLYHERBAL EXTRACT GELS ON THE PERCENTAGE OF HAIR GROWTH IN DIFFERENT PHASES

TABLE 5: EFFECT OF POLYHERBAL EXTRACT GELS ON THE PERCENTAGE OF HAIR GROWTH IN DIFFERENT PHASES

S. no.	Group	After 10 days		After 20 days		After 30 days	
		Anagen	Telogen	Anagen	Telogen	Anagen	Telogen
1	Control	37±0.33	69±1.20	45±0.47	65±1.40	51±0.49	60±1.20
2	Standard (2% Minoxidil)	43±0.34	58±0.70	58±0.61	51±0.40	65±0.43	43±0.50**
3	PG1	42±0.58	60±0.80	56±0.51	52±0.60	63±0.43	45±0.35**
4	PG2	39±0.51	66±1.01	48±0.50	62±1.20	54±0.52	56±0.40*

All Values were expressed as mean ± SEM, n = 6 *P < 0.05 and **P < 0.01: Significance versus control.

Hair Length Measurement: The length of hair was measured and observed that in group Control, Standard (2% Minoxidil), PG1, PG2 was found to be 9 ± 0.55 mm, 16 ± 0.80 mm, 15 ± 0.60 mm and 11 ± 0.60 mm respectively. Thus, both polyherbal extract gel formulations in treated groups showed significant growth as compared to control. The results are summarized in **Table 6**.

TABLE 6: EFFECT OF POLYHERBAL EXTRACT GELS ON HAIR LENGTH AFTER 30 DAYS OF APPLICATION IN RATS

S. no.	Group	Hair Length (mm)
1	Control	9±0.55
2	Standard (2% Minoxidil)	16±0.80**
3	PG1	15±0.60**
4	PG2	11±0.60*

All Values were expressed as mean ± SEM, n = 6 *P < 0.05 and **P < 0.01: Significance versus control.

Hair Weight Measurement: The weight of newly grown hairs in the treated groups and standard group were measured and compared with the control group. The weight of hairs was significantly increased in the treated groups of polyherbal extract gels *i.e.* PG1, PG2 found to be 59 ± 1.10 mg/cm^2, 52 ± 0.90 mg/cm^2, respectively. Whereas Standard (2% Minoxidil) was found to be 60 ± 1.20 mg/cm^2 area of dorsal skin of rat. The results are summarized in **Table 7**.

TABLE 7: EFFECT OF POLYHERBAL EXTRACT GELS ON HAIR WEIGHT AFTER 30 DAYS OF APPLICATION IN RATS

S. no.	Group	Weight of hair (mg/cm^2)
1	Control	48±2.70
2	Standard (2% Minoxidil)	60±1.20**
3	PG1	59±1.10**
4	PG2	52±0.90*

All Values were expressed as mean ± SEM, n = 6 *P < 0.05 and **P < 0.01: Significance versus control.

***In-vitro* Hair Follicle Culture:** As shown in **Table 8**, the growth of hair follicles was observed under binocular microscope at higher magnification. The polyherbal extract gel formulations *i.e.* PG1, PG2 in treated groups showed a significant increase in length over 72 h in culture was found to be 0.26 ± 0.02 mm, 0.13 ± 0.03 mm, respectively. Whereas Standard (2% Minoxidil) was found to be 0.27 ± 0.04 mm.

TABLE 8: EFFECT OF POLYHERBAL EXTRACTS GELS ON *IN-VITRO* HAIR FOLLICLE CULTURE

S. no.	Group	Increase in hair follicle length (mm) ± S.D.	
		After 24 h.	After 72 h.
1	Control	0.04±0.03	0.09±0.02
2	Standard (2% Minoxidil)	0.13±0.04	0.27±0.04**
3	PG1	0.12±0.04	0.26±0.02**
4	PG2	0.07±0.02	0.13±0.03*

All Values were expressed as mean ± SEM, n = 6 *P < 0.05 and **P < 0.01: Significance versus control

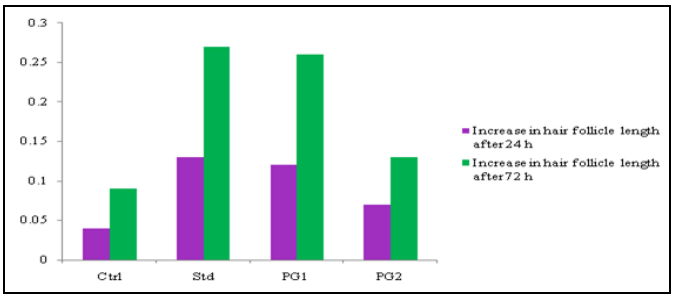

FIG. 5: EFFECT OF POLYHERBAL EXTRACT GELS ON *IN-VITRO* HAIR FOLLICLE CULTURE

The polyherbal extract gel formulation on topical application stimulates the hair growth initiation and hair completion time. It was observed that the hair growth initiated from the shaved area at the start of the 1st week in Standard (2% Minoxidil) and PG1 group and 2nd week in PG2 group and 3rd week in control group and in the whole denuded area has been covered at the end of the course in comparison to the control. This may be due to the gentle rubbing of the shaved skin while applications of polyherbal extract gel formulation. This enhances the blood circulation in the local area. Thus, it may exert some effect on hair growth.

The groups treated with polyherbal extract gel produced a greater effect on the length of hair as compared to control group. This may be due to the

premature switching of hair follicles from telogen phase to the anagen phase of the hair growth cycle. The *in-vitro* study revealed that polyherbal extract gel has a direct impact on hair follicles and thus may improve hair growth.

CONCLUSION: In the present investigation, Carbopol 934 and HPMC K100 based polyherbal extract gel formulations were developed and their hair growth activity along with physicochemical parameters were evaluated. In order to find out marketing potential, all the formulations were compared with 2% Minoxidil for similarity.

The findings from the above activities revealed that polyherbal extract gel formulation PG1 is feasible with Carbopol 934 polymer.

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CONFLICTS OF INTEREST: Authors declare no conflict of interest.

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